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Lateral parabrachial lesions impair taste aversion learning induced by blood-borne visceral stimuli

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Abstract

The lateral parabrachial area (LPB), main relay from the area postrema (AP), plays a role in processing visceral information and is thus of potential importance in taste aversion learning (TAL). This study used a lesion approach to address whether LPB functional relevance depends upon the features of toxins that serves as visceral stimuli in TAL. In addition, we explored whether LPB involvement in TAL is restricted to those toxic events detected by the AP or whether it has a more general role. Results showed that LPB-lesioned animals were disrupted in acquiring a TAL induced by blood-borne AP-dependent aversive stimuli (intraperitoneal methylscopolamine) and by AP-independent stimulus (intraperitoneal ethanol), but still, clearly developed strong aversions when intragastric hypertonic sodium chloride, a vagally processed aversive stimulus, served as the aversive stimulus. These findings suggest that the LPB plays a critical role in TAL induced by blood-borne toxins, such as methylscopolamine or ethanol, but is not necessary for vagally mediated stimulus, such as sodium chloride. The present results are discussed in the context of the hypothesis holding separable and independent neural systems underlying TAL.

Keywords: Lateral parabrachial area; Taste aversion learning; Visceral processing; Blood-borne toxins; Vagal-mediated toxins

1. Introduction

Conditioned taste aversion learning (TAL) is a very special kind of learning consisting in the avoidance of a gustatory/flavored stimulus previously paired with gastric malaise. The neural basis for this learning still remains controversial, probably due to important differences in the experimental procedures employed to induce TAL. Understanding associative mechanisms in TAL must be done through the analysis of neural pathways involved in both gustatory and visceral processing (Chambers, 1990; Garcia et al., 1985).

It has been pointed out that the way by which information from the visceral stimulus is conveyed to the brain varies, among others, with the particular chemical characteristics of the aversive drugs (Arnedo et al., 1990; Boissonade and Davison, 1996; Borison, 1974; Coil et al., 1978; Cubero et al., 1999; Chambers, 1990; Goehler et al., 1995; Ishizuka et al., 1997; Kamoto et al., 1993; Kiefer et al., 1980; Martin et al., 1978; Monnikes et al., 1997; Peele et al., 1986; Rabin et al., 1987). It has been previously proposed that independent and nonredundant cerebral systems underlie visceral processing as well as TAL acquisition (Arnedo, 1987; Arnedo et al., 1990, 1991, 1993; Coil et al., 1978; Chambers, 1990; Mediavilla et al., 1998; Schafe et al., 1998). A distinction has been noted between TAL in response to visceral treatments (e.g., intragastric copper sulfate or hypertonic sodium chloride), which depend entirely on an intact vagus nerve (Arnedo, 1987; Arnedo et al., 1990, 1991, 1993; Coil et al., 1978), and TAL in response to blood-borne toxins (e.g., intraperitoneal LiCl or methylscopolamine), which depend on the area postrema (AP) (Berger et al., 1973; Bernstein et al., 1992; Borison, 1974; Gallo et al., 1990; Ritter et al., 1980) and are unaffected by vagotomy (Arnedo et al., 1990). Consistent with that distinction, we have reported that peripheral vagotomy (Arnedo et al., 1991) and central axotomy of vagal afferent to the nucleus of the solitary tractus (NTS) (Arnedo et al., 1990, 1993) impair the

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acquisition of intragastric sodium chloride-induced TAL but not a delayed intraperitoneal lithium-induced TAL (Arnedo et al., 1993). In contrast, AP lesion impairs a delayed intraperitoneal lithium-induced TAL (Agüero et al., 1993b) or methylscopolamine-induced TAL (Gallo et al., 1990) but has no effect on an intragastric hypertonic sodium chloride- (Arnedo et al., 1990) or intragastric copper sulfate-induced TAL (Coil and Norgren, 1981; Coil et al., 1978). Despite the fact that AP is critically involved in toxin detection (Borison, 1974; Miller and Rugiero, 1994; Miller et al., 1994; Monnikes et al., 1997), not all blood-borne drugs depend on AP to support TAL (e.g., amphetamine and ethanol) (Hunt, 1996; Rabin et al., 1987; Stewart et al., 1988).

The lateral parabrachial region (LPB) is the main relay from the AP (Cunningham et al., 1994; Fullwiler and Saper, 1984; van der Kooy and Koda, 1983). In this regard, electrophysiological (Kobashi et al., 1993; Papas and Ferguson, 1990; Suemori et al., 1994), molecular (Sakai and Yamamoto, 1997; Swank and Bernstein, 1994; Swank et al., 1995; Thiele et al., 1996; Yamamoto et al., 1992a,b), and behavioral evidence (Agüero et al., 1992a,b; Cubero and Puerto, 2000; Reilly, 1999; Reilly and Trifunovic, 1999, 2000; Sakai and Yamamoto, 1998) suggest a role for the LPB in visceral processing and TAL induced by blood-borne toxic stimuli. Previous work reports that ibotenic acid or electrolytic lesions centered in the LPB abolish acquisition but not retention of a lithium-induced TAL (Agüero et al., 1993b; Sakai and Yamamoto, 1998), delayed methylscopolamine-induced TAL (Cubero and Puerto, 2000), and disrupt the TAL acquisition induced by electrical stimulation of the AP when paired with a flavored stimulus (Agüero et al., 1993a). In addition, it has been reported that strong c-Fos expression is seen in LPB (Sakai and Yamamoto, 1997; Swank et al., 1995; Thiele et al., 1996) when a range of drugs is delivered intraperitoneally, including drugs such as amphetamine (Swank et al., 1995) or ethanol (Sakai and Yamamoto, 1997; Stewart et al., 1988), which are apparently not processed by the AP during TAL. Therefore, the LPB plays a role in processing visceral blood-borne information and is thus of potential importance in TAL. Whether its functional relevance is restricted to those toxic events detected by the AP (e.g., lithium chloride, methylscopolamine) or whether it has a more general role in TAL remains to be determined. This question has not been addressed using a lesion approach. The main issue in this paper is to analyze deeper whether the LPB is necessary in TAL acquisition depending upon the features of the aversive stimulus employed. For that purpose, groups of animals lesioned in the LPB will be trained in a TAL task induced by blood-borne vs. vagally mediated toxins (intragastric hypertonic sodium chloride). In addition, the present study will explore the LPB functional relevance in processing AP-dependent (intraperitoneal methylscopolamine) and AP-independent (intraperitoneal ethanol) visceral aversive stimuli.

2. Method

2.1. Animals

Wistar rats obtained from the University of Granada (Spain), weighing 275–350 g, were individually housed in methacrylate cages, which also served as experimental training cages. Rats were maintained in a 12/12-h light–dark cycle with free access to water and food except as noted otherwise. All the manipulations were conducted during the light phase. All behavioral procedures as well as surgical and pharmacological techniques were conducted in agreement with the animal care guidelines established by the Spanish Royal Decree 223/1988.

2.2. Apparatus

Experimental procedures were conducted in methacrylate chambers measuring $30 \times 15 \times 30$ cm, with two orifices located at the same height and distance from the midline. Through those orifices, the animal had access to spouts attached to graduated burettes through which flavors and water were delivered. The animals were lesioned using a Stoelting stereotaxic instrument (Model 51.600) and a Cibertec electrolytic lesion maker, which supplied direct negative current through a monopolar electrode, which was approximately 200 μm in diameter and was insulated throughout its length except for the last 0.5 mm.

2.3. Surgery

Surgery was performed under general anesthesia with sodium thiobarbital (50 mg/kg, Lab. Abbot, Spain). The animals were placed in the head holder and two small threpine holes were bilaterally drilled at the anatomical coordinates obtained from Paxinos and Watson's (1986) stereotaxic atlas according to the interaural point of reference: AP = − 0.16 mm, L = ± 2.4 mm, and V = 3.4 mm. The electrode was positioned in the LPB and a 1.5-mA current passed through it during 20 s. The sham-lesioned group received identical surgical procedure except no current was administered. At the end of the surgical procedure, the electrode was removed and the wound sutured. After the surgery, animals in the Experiment 2 were implanted with a Silastic intragastric fistula (Silastic medical grade tubing, Dow Corning, MI) that allowed direct manipulations to the gastric cavity. During the recovery period, the fistula was flushed everyday with distilled water to avoid clogging.

2.4. Behavioral procedure

2.4.1. Experiment 1: TAL induced by intraperitoneal methylscopolamine and ethanol

Animals were randomly assigned to one of two basic groups (LPB-lesioned and sham-lesioned). Half of the animals in each group received, as an aversive stimulus, intra-

	DAY 1	DAY 2	DAY 3
HALF OF SUBJECTS	FLAVOR 1 + DRUG (paired condition)	FLAVOR 2 + SALINE (unpaired condition)	FREE CHOICE TEST
HALF OF SUBJECTS	FLAVOR 1 + SALINE (unpaired condition)	FLAVOR 2 + DRUG (paired condition)	FREE CHOICE TEST

Fig. 1. Diagram showing the balanced experimental conditions in the acquisition of the TAL task. On Day 1, flavor 1 + drug represents for half of the animals, the paired session in which the flavor is associated to the aversive stimulus. Flavor 1 + saline represents the unpaired session for the rest of the animals. On Day 2, there is a reversal of the experimental conditions and a second flavor is presented. Day 3 is a two-bottle free-choice test, in which both flavored solutions 1 and 2 were simultaneously offered for 10 min.

peritoneal methylscopolamine (MSP/IP), and the remaining half, intraperitoneal ethanol (E/IP). Thus, four groups were trained in TAL, two of them LPB-lesioned [PBX-MSP/IP ($n = 9$) and PBX-E/IP ($n = 12$)] and two of them sham-lesioned [SHAM-MSP ($n = 10$) and SHAM-E/IP ($n = 8$)]. The behavioral procedure started 1 week postoperatively, once the animals recovered from their surgical procedure. During the pretraining session all the animals were water deprived for 23 h and 50 min and allowed to drink tap water 10 min/day from two graduated burettes presented simultaneously (to avoid position preferences) through the frontal holes in the cages. After 4 days of pretraining with water, the experimental procedure began as shown in Fig. 1. The learning procedure consisted of one-bottle acquisition TAL procedure. On Day 1, the animals were presented for 10 min with one of two possible flavored solutions: 0.5% strawberry (S) or coconut (C) extract (McCormick, Baltimore, MD) diluted in water. Half of the animals in each basic group (LPB-lesioned; sham-lesioned) received intraperitoneally one of two aversive stimuli (paired condition): methylscopolamine (1 mg/kg, Sigma, Madrid, Spain) dissolved in distilled water in a concentration of 1 mg/ml (groups PBX-MSP/IP and SHAM-MSP/IP), or ethanol (1.5 g/kg) in a concentration of 20% v/w dissolved in distilled water (PBX-E/IP and SHAM-E/IP groups). The remaining half of the animals received intraperitoneal isotonic saline injection (unpaired condition), matching drug volume for paired condition. The time to deliver the aversive compound in this study was selected in order to match experimental conditions in Experiments 1 and 2. We have shown that in order to successfully develop a sodium-induced TAL through vagal processing, the ingestion from flavors must simultaneously be paired to the aversive compound (Arnedo, 1987; Arnedo et al., 1990, 1991, 1993). Therefore, we decided to deliver all the aversive compounds in this study once the animal had consumed 2 ml from the flavor. After delivering the visceral stimulus, the animals would return to their experimental cages to complete a total of 10-min drinking from the flavored solution.

On Day 2, 24 h later, the second flavored solution was presented and the same procedure as described for Day 1 was repeated. The sequence of the experimental conditions were properly balanced in such a way that solution pairings were reversed. After that, all the animals had experienced both flavored solutions, but only one of them had been paired in a single trial, to the aversive stimuli (paired condition) (see Fig. 1).

2.4.2. Experiment 2: TAL induced by intragastric hypertonic sodium chloride

Animals were divided into two groups, one damaged in LPB, PBX-Na/IG ($n = 7$), and one sham-lesioned group, SHAM-Na/IG ($n = 10$). TAL procedure was identical to that

Fig. 2. Schematic unilateral representation of a sequence of brain coronal sections of the bilaterally induced lesions in the LPB. The lesions extended approximately from 9.3 to 8.8 posterior to bregma. The schematic representation was adapted from Paxinos and Watson (1986). The darkened area represents the smallest lesion and the gray area, the largest lesion.

in the Experiment 1 (Fig. 1) except that the aversive stimulus was 5 ml of 5% hypertonic sodium chloride dissolved in distilled water and intragastrically delivered at a rate of 1.5 ml/min. In the unpaired condition, the animals received an intragastric isotonic saline injection matching drug volume with the paired condition. On Day 2, there was a reversal of the experimental conditions, as shown in Fig. 1.

2.5. Testing

After training, all the animals were tested for TAL acquisition. A two-bottle free-choice test was conducted on Day 3 by simultaneously placing two burettes, each one containing one of the two flavored solutions previously used during the training sessions. In this test phase, animals were allowed to freely drink for 10 min and the total amount ingested was recorded.

2.6. Histology

After concluding the behavioral experiments, the animals were deeply anesthetized with an overdose of sodium thiobarbital and intracardially perfused with isotonic saline and 4% formaldehyde solution. The brains were removed and stored in formaldehyde for at least 48 h. Then the brains were cut with a freezing microtome in coronal 40-μm sections. Placement of electrolytic lesions was verified with a Cresyl violet staining under light microscope. In all of the animals, electrolytic lesions were centered in the LPB area (see Fig. 2) with minimum damage, if any, to the brachium or the medial parabrachial area. Lesions expanded, as average, approximately 8.6–9.5 mm posterior to bregma, 1.5–2.5 mm lateral to medial line, and 6–7.2 mm ventral to the skull surface, according to Paxinos and Watson (1986).

3. Results

3.1. Experiment 1: Effects of LPB lesions on intraperitoneal ethanol- and methylscopolamine-induced TAL

Data from the two-bottle choice test were analyzed with two-way, $2 \times 2 \times 2$ (Group × Aversive Stimuli × Flavored Solution), general analysis of variance (ANOVA), with two between-group factors (group and aversive stimuli) and one within-subject factor (flavored solution). The factor group examined differences between lesion and control sham-lesioned groups, the factor aversive stimuli evaluated consumption differences between groups receiving each toxin (ethanol and methylscopolamine), whereas the within-subject factor flavored solution contrasted intake of paired vs. unpaired flavored solution. TAL was indicated when consumption of the paired solution was significantly lower than the consumption of the unpaired solution during the two-bottle choice test. The general ANOVA revealed a non-significant effect for the factor group [$F(1,35) = 0.1$, $P = .7$] and for the factor aversive stimuli [$F(1,35) = 1.8$, $P = .1$]. Also, the interaction Group × Aversive Stimuli was not significant [$F(1,35) = 0.4$, $P = .5$] (see Fig. 3). These data indicated that the total amount of solution ingested in the choice test were similar in both the control and the lesioned groups, when either ethanol or methylscopolamine served as aversive stimulus. Taken together, these results confirm that all animals, despite LPB lesions or type of drug injected to elicit TAL, are capable of normal drinking and exhibit nonaltered motivational or motor behaviors.

However, the general ANOVA revealed a different pattern of consumption on the test day for control and lesioned animals, showing a significant effect for the within-subject factor, flavored solution [$F(1,35) = 20.7$, $P < .0001$], as well as for the interaction effect Group × Flavored Solution [$F(1,35) = 25.8$, $P < .00000$]. Independent ANOVAs were conducted to further analyze these effects by comparing consumption of paired and unpaired solution during the choice test. They showed that both stimuli, intraperitoneal methylscopolamine and ethanol, served as aversive stimuli in TAL, eliciting clear avoidance of the paired flavor in sham control groups [SHAM-MSP/IP group, $F(1,9) = 53.5$, $P < .0001$, and SHAM-E/IP group, $F(1,7) = 27.2$, $P < .001$]. In contrast, animals lesioned in the LPB were severely disrupted in TAL when methylscopolamine (PBX-MSP/IP) or ethanol (PBX-E/IP) served as the visceral stimuli, drinking similar amounts of both paired and unpaired flavored solution on the test day [$F(1,8) = 1.5$, $P = .2$, and $F(1,11) = 1.9$, $P = .1$, respectively].

Thus, the present data confirm previous results suggesting that the LPB is necessary for TAL to be acquired and/or expressed when blood-borne compounds, such as intraperitoneal methylscopolamine or ethanol, serve as the visceral stimuli.

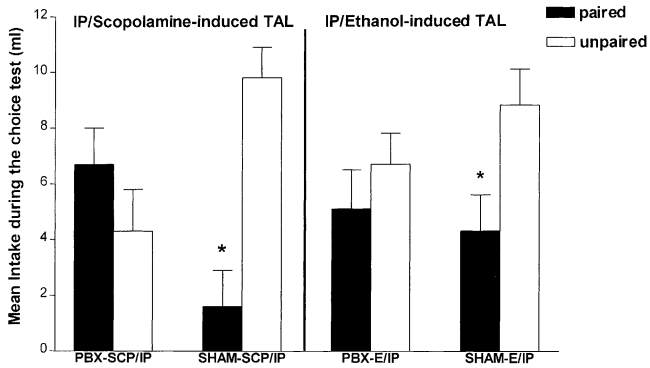

Fig. 3. Mean (±S.E.M.) consumption during the choice test by LPB-lesioned or sham-lesioned subjects in Experiment 1 previously trained in intraperitoneal methylscopolamine (PBX-MSP/IP, SHAM-MSP/IP)- or ethanol (PBX-E/IP, SHAM-E/IP)-induced TAL. The paired stimulus represents the intake of the flavor associated with visceral malaise and the unpaired stimulus represents the intake of the flavor associated with saline in the TAL acquisition stage. * $P < .01$ paired relative to unpaired.

3.2. Experiment 2: Effects of LPB lesions in TAL induced by intragastrically delivered hypertonic sodium chloride

Data from choice test were analyzed with a 2×2 (Group × Flavored Solution), two-way ANOVA, with a between-group factor (group) that evaluated differences between lesioned and sham groups, and a within-subject factor (flavored solution), that measured and compared the total consumption associated with the paired vs. unpaired solution in the choice test. The ANOVA revealed a nonsignificant effect both in the group factor [$F(1,15) = 0.1$, $P = .7$] and interaction Group × Flavored Solution factor [$F(1,15) = 0.3$, $P = .5$] (see Fig. 4). These data showed that the total amount consumed for the lesioned and sham-lesioned animals was similar which, once again, suggest no impairments in motor and motivational function in lesioned animals. Finally, the within-subject factor, flavored solution, attained statistical significance [$F(1,15) = 15.6$, $P < .001$], showing that both parabrachial-lesioned and sham animals acquired a TAL task by avoiding the flavor previously paired to hypertonic sodium chloride.

The pattern of results emerging in sodium-induced TAL was clearly opposite of that for methylscopolamine- or ethanol-induced TAL. In order to evaluate if animals developed a sodium-induced TAL due to differences in the strength of the aversion elicited by each toxin, data from choice test of sham groups were conjointly analyzed. A two-way ANOVA with a between-subject factor aversive stimuli assessing differences between methylscopolamine-, ethanol- and sodium-injected animals and a within-subject factor, flavored solution, which evaluated intake of paired vs. unpaired solution during the choice test, was conducted. The ANOVA showed nonsignificant differences between groups [$F(2,25) = 0.8$, $P = .4$] and in the interaction of Flavored Solution × Aversive Stimuli [$F(1,25) = 1.5$, $P = .2$]. Finally, the factor flavored solution attained statistical significance [$F(1,25) = 43.2$, $P = .00000$]. Those results suggest that, at the chosen dose, all of the drugs employed in this TAL study, intragastric sodium chloride, intraperitoneal methylscopolamine, and intraperitoneal ethanol, triggered a comparable pattern of behavioral aversion. However, as described in the Experiment 1, only the TAL induced by blood-borne mediated drugs (methylscopolamine and ethanol) were dependent on the LPB integrity.

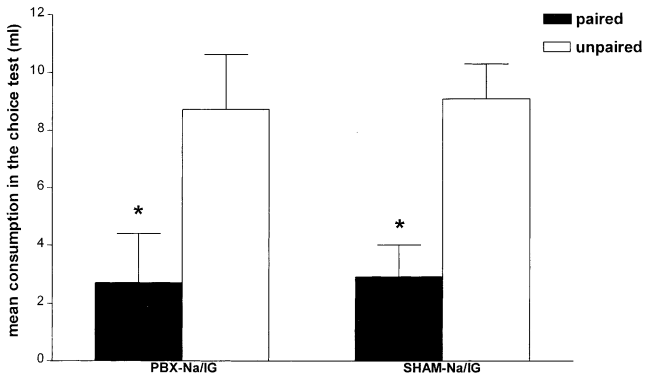

Fig. 4. Mean (±S.E.M.) consumption during the choice test by LPB-lesioned (PBX-Na) and sham-lesioned subjects (SHAM-Na) in Experiment 2 previously trained in a hypertonic sodium chloride-induced TAL. Paired stimulus represents the intake of the flavor associated with visceral malaise and unpaired stimulus represents the intake of the flavor associated with saline in the TAL acquisition stage. * $P < .01$ paired relative to unpaired.

4. Discussion

These studies indicate that TAL impairments induced by lesions centered in the LPB depend upon the nature of the visceral aversive stimulus. LPB-lesioned animals were disrupted in an intraperitoneal methylscopolamine- and ethanol-induced TAL but still, clearly developed strong aversions when intragastric hypertonic sodium chloride served as the aversive stimulus. This latter effect indicates intact gustatory as well as associative capacities in LPB-lesioned animals, suggesting that TAL impairments seen after LPB lesions in Experiment 1 were not related to sensory impairments or nonspecific effects of the lesion. Moreover, the fact that all of the aversive compounds in this study (methylscopolamine, ethanol, and hypertonic sodium chloride) elicited similar patterns of aversion suggests that the deficits seen in LPB-damaged animals given different aversive treatments were not due to differences in the strength of visceral aversion induced by the drugs employed as aversive stimulus.

The present study was aimed at evaluating, through a lesion approach, whether the LPB is pivotal for visceral processing. It has been claimed in TAL research that several factors such as the nature of visceral toxin, the route employed to deliver it, the response requirements, or the temporal parameters in experimental procedures, constrain cerebral systems underlying visceral processing and TAL (Arnedo et al., 1993; Cubero et al., 1999; Chambers, 1990; Mediavilla et al., 1998; Schafe et al., 1998). Consistent with this hypothesis, we have previously proposed, at least two independent visceral processing routes, one vagally mediated and one blood-borne mediated. In this context, subdiaphragmatic vagotomy (Arnedo et al., 1991) or central vagal axotomy (Arnedo et al., 1990, 1993) disrupts flavor aversions to intragastric hypertonic sodium in a concurrent short-term TAL task, which seems to involve vagal function. However, these vagal manipulations have no effect on a sequential-delayed TAL procedure when a blood-borne compound, intraperitoneal lithium, serves as the aversive stimuli (Arnedo, 1987). Meanwhile, AP lesions disrupt a delayed intraperitoneal methylscopolamine- (Berger et al., 1993; Gallo et al., 1990) or lithium chloride-induced TAL (Ritter et al., 1980) but not a TAL induced by concurrent intragastric hypertonic sodium chloride (Arnedo et al., 1990). Moreover, we had reported that lesions centered in the LPB, main relay from AP (Cunningham et al., 1994), impair TAL acquisition to intraperitoneal lithium chloride (Agüero et al., 1993b) and methylscopolamine (Cubero and

Puerto, 2000) in a sequential-delayed TAL task. In addition to behavioral work, molecular studies have also pointed to the central role played by the LPB in processing blood-borne mediated toxin. It has been reported that strong c-Fos is seen in LPB (Sakai and Yamamoto, 1997; Swank and Bernstein, 1994; Thiele et al., 1996) when a range of drugs is delivered intraperitoneally including drugs such as amphetamine or ethanol, for which AP seems to be unnecessary during TAL (Hunt, 1996; Rabin et al., 1987; Stewart et al., 1988). Therefore, our findings can be interpreted in accordance with the hypothesis that holds independent and nonredundant cerebral circuits supporting acquisition and/or expression of TAL under different experimental requirements (Arnedo et al., 1990; Cubero et al., 1999; Mediavilla et al., 1998; Schafe et al., 1998). In this regard, the present study suggests that the LPB is specifically involved in processing blood-borne but not vagally dependent stimuli, but also indicates that that the role of this pontine area in TAL is not limited to AP-dependent treatments since ethanol-induced TAL appears to depend on an intact LPB but not on the AP (Hunt, 1996; Kiefer et al., 1980; Stewart et al., 1988). The visceral malaise and the TAL induced by delivering intragastrically hypertonic sodium chloride, do not rely upon the LPB since animals with damage to this region still developed TAL in Experiment 2. In this latter case, illness elicited by intragastric sodium would have to be coded probably by gastric receptors, which convey a vagal message of toxicity and visceral distress to the central nervous system (Arnedo, 1987; Arnedo et al., 1990, 1991, 1993; Mei, 1983; Mei and Garnier, 1986).

Consistent with the hypothesis holding separable and independent neural systems underlying TAL, the pattern of results obtained in LPB-lesioned animals suggests a selective disruption of visceral and/or associative processing for blood-borne toxins but not for vagal-mediated aversive stimuli inducing TAL. With the present findings, we cannot rule out the LPB involvement in sodium-induced TAL. Nonetheless, we suggest that whether LPB is necessary for the acquisition and/or expression of the learned response depends on the nature of the aversive visceral stimulus employed.

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References

Agüero A, Arnedo M, Gallo M, Puerto A. Learning of the parabrachial nuclei disrupt aversion learning induced by electrical stimulation of the area postrema. Brain Res Bull 1993a;30:585–92.

Agüero A, Arnedo M, Gallo M, Puerto A. The functional relevance of the lateral parabrachial nucleus in lithium chloride-induced aversion learning. Pharmacol, Biochem Behav 1993b;45:973–8.

Arnedo M. Bases biológicas del aprendizaje interoceptivo: una disociación comportamental de los sistemas periféricos implicados en el procesamiento visceral. Doctoral dissertation, Universidad de Granada, 1987.

Arnedo M, Gallo M, Agüero A, Puerto A. Effects of medullary afferent vagal axotomy and area postrema lesions on short-term and long-term NaCl-induced taste aversion learning. Physiol Behav 1990; 47:1067–74.

Arnedo M, Gallo M, Agüero A, Puerto A. Differential effects of subdiaphragmatic vagotomy on ClNa induced aversion learning. Behav Neural Biol 1991;55:141–53.

Arnedo M, Gallo M, Agüero A, Molina F, Puerto A. Medullary afferent vagal axotomy disrupts NaCl induced short-term taste aversion learning. Behav Neural Biol 1993;59:69–75.

Berger B, Wise D, Stein C. Area postrema damage and bait shyness. J Comp Physiol Psychol 1973;82:475–9.

Bernstein LI, Chavez M, Allen D, Taylor EM. Area postrema mediation of physiological and behavioral effects of lithium chloride in the rats. Brain Res 1992;575:132–7.

Boissonade FM, Davison JS. Effects of vagal and splanchnic nerve transection on fos expression in ferret brain stem after emetic stimuli. Am J Physiol 1996;40:R228–36.

Borison HL. Area postrema: chemoreceptor trigger zone for vomiting. Is that all? Life Sci 1974;14:1807–17.

Chambers KC. A neural model for conditioned taste aversion. Annu Rev Neurosci 1990;13:273–385.

Coil JD, Norgren R. Taste aversion conditioned with intravenous copper sulphate: attenuation by ablation of the area postrema. Brain Res 1981; 212:425–33.

Coil JD, Rogers R, García J, Novin D. Conditioned taste aversion: vagal and circulatory mediation of the toxic unconditioned stimulus. Behav Biol 1978;24:509–19.

Cubero I, Puerto A. Lateral parabrachial lesions impair intraperitoneal but not intraventricular methylscopolamine-induced taste aversion learning. Brain Res 2000;871(1):113–9.

Cubero I, Thiele T, Bernstein I. Insular cortex lesions and taste aversion learning: effects of conditioning method and timing of lesion. Brain Res 1999;839:323–30.

Cunningham E, Miselis R, Sawchenco P. The relationship of efferents projections from the area postrema to vagal motor and brain stem catecholamine containing cell groups: an axonal transport and immunohistochemical study in the rat. Neuroscience 1994;58: 635–48.

Fullwiler C, Saper C. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res Rev 1984;7:229–59.

Gallo M, Arnedo M, Agüero A, Puerto A. The functional relevance of the area postrema in drug induced aversion learning. Pharmacol, Biochem Behav 1990;35:543–51.

Garcia J, Lasiter P, Bermudez-Rattoni F, Deems D. A general theory of aversion learning. In: Braveman NS, editor. Experimental assessments and clinical implications of conditioned food aversion. Ann NY Acad Sci 1985;443:8–22.

Goehler LE, Busch CR, Tartaglia N, Relton J, Sisk D, Maier SF, Watkins LR. Blockade of cytokine induced conditioned taste aversion for vagal subdiaphragmatic vagotomy: further evidence for vagal mediation of immune-brain communication. Neurosci Lett 1995;185:163–6.

Hunt WA. Role of acetaldehyde in the actions of ethanol on the brain — a review. Alcohol 1996;13:147–51.

Ishizuka Y, Ishida Y, Kunitake T, Kato K, Hanamori T, Mitsuyama Y, Kannan H. Effects of area postrema lesion and abdominal vagotomy on interleukin-1β-induced norepinephrine release in the hypothalamic paraventricular nucleus region in the rat. Neurosci Lett 1997;223:57–60.

Kamoto TH, Ito Y, Nagakura A, Yuki H, Yamano M, Miyata K. Mechanisms of cisplatin- and *m*-chlorophenylbiguanide-induced emesis in ferrets. Eur J Pharmacol 1993;238:369–76.

Kiefer S, Cabral R, Rusiniak K, Garcia J. Ethanol-induced flavor aversions

in rats with subdiaphragmatic vagotomy. Behav Neural Biol 1980; 29:246–54.
Kobashi M, Ichikawa H, Sugimoto T, Adachi A. Response of neurons in the solitary tract nucleus, area postrema and lateral parabrachial nucleus to gastric load of hypertonic saline. Neurosci Lett 1993;158: 47–50.
Martin JR, Cheng FY, Novin D. Acquisition of learned taste aversion following bilateral subdiaphragmatic vagotomy in rats. Physiol Behav 1978;21:13–7.
Mediavilla C, Molina F, Puerto A. Bilateral lesions in the cerebellar interpositus–dentate region impair taste aversion learning. Physiol Behav 1998;65:25–33.
Mei N. Recent studies on intestinal vagal afferent innervation. Functional implications. J Auton Nerv Syst 1983;9:199–206.
Mei NY, Garnier L. Osmosensitive vagal receptors in the small intestine of the cat. J Auton Nerv Syst 1986;16:159–70.
Miller AD, Rugiero DA. Emetic reflex arc revealed by expression of the immediate-early gene c-Fos in the cat. J Neurosci 1994;12(2):871–88.
Miller AD, Nonaka S, Jakins J. Brain areas essential or non-essential for emesis. Brain Res 1994;647:255–64.
Monnikes H, Lauer G, Bauer C, Tebbe J, Zittel TT, Arnold R. Pathways of Fos expression in locus ceruleus, dorsal vagal complex, and PVN in response to intestinal lipid. Am J Physiol 1997;273(6 Pt 2):2059–71.
Papas S, Ferguson AV. Electrophysiological characterization of reciprocal connections between the parabrachial nucleus and the area postrema in the rat. Brain Res Bull 1990;24:577–82.
Paxinos G, Watson C. The rat brain in stereotaxic coordinates 2nd ed. New York: Academic Press, 1986.
Peele DB, Mcphai RC, Farmer JD. Flavor aversions induced by thallium sulfate; importance of route of administration. Neurobehav Toxicol Teratol 1986;8:273–7.
Rabin B, Hunt W, Lee J. Interactions between radiation and amphetamine in taste aversion learning and the role of the area postrema in amphetamine-induced conditioned taste aversion. Pharmacol, Biochem Behav 1987;27:677–83.
Reilly S. The parabrachial nucleus and conditioned taste aversion. Brain Res Bull 1999;48:239–50.
Reilly S, Trifunovic R. Lateral parabrachial nucleus lesions in the rat: aversive and appetitive gustatory conditioning. Brain Res Bull 1999;52:269–78.
Reilly S, Trifunovic R. Lateral parabrachial nucleus lesions in the rat: long- and short duration gustatory preference tests. Brain Res Bull 2000; 51:177–86.
Ritter S, McGlone J, Kelley K. Absence of lithium-induced taste aversion after area postrema lesion. Brain Res 1980;201:501–6.
Sakai N, Yamamoto T. Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various UCS. NeuroReport 1997;8:2215–20.
Sakai N, Yamamoto T. Role of the medial and lateral parabrachial nucleus in acquisition and retention of conditioned taste aversion in rats. Behav Brain Res 1998;93:63–70.
Schafe GE, Thiele TE, Bernstein IL. Conditioning method dramatically alters the role of amygdala in taste aversion learning. Learn Mem 1998;5:481–92.
Stewart RB, Perlanski E, Grupp LA. Area postrema and alcohol: effects of area postrema lesions on ethanol self-administration, pharmacokinetics, and ethanol-induced conditioned taste aversion. Alcohol: Clin Exp Res 1988;12:698–704.
Suemori K, Kobashi M, Adachi A. Effects of gastric distension and electrical stimulation of dorsomedial medulla on neurons in parabrachial nucleus of rats. J Auton Nerv Syst 1994;48:221–9.
Swank M, Bernstein IL. c-fos induction in response to a conditioned stimulus after single trial taste aversion learning. Brain Res 1994; 636:202–8.
Swank MW, Schafe GE, Bernstein IL. c-fos induction in response to taste stimuli previously paired with amphetamine or lithium chloride during taste aversion learning. Brain Res 1995;673:226–51.
Thiele TE, Roitman MT, Bernstein IL. c-fos induction in rat brainstem in response to ethanol and lithium chloride induced conditioned taste aversion. Alcohol: Clin Exp Res 1996;20:1023–8.
van der Kooy D, Koda L. Organization of the projections of a circumventricular organ: the area postrema in the rat. J Comp Neurol 1983;219:328–38.
Yamamoto T, Shimura T, Azuma S, Bai WZh, Fjimoto S. Induction of c-fos-like protein in the central nervous system of the rat following internal malaise. In: Inoki R, Shigenaga Y, Tohyama M, editors. Processing and inhibition of nociceptive information. Amsterdam: Elsevier, 1992a. pp. 165–8.
Yamamoto T, Shimura T, Sako N, Azuma S, Bai W, Wakisaka S. c-fos expression in the rat brain after ip injection of lithium chloride. NeuroReport 1992b;3:1049–52.